Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 1187–1191, February 1991
Microbiology

Tyrosine phosphate hydrolysis of host proteins by an essential *Yersinia* virulence determinant

(*yopH*/phagocytosis/macrophage)

JAMES B. BLISKA*, KUNLIANG GUAN†, JACK E. DIXON†, AND STANLEY FALKOW*

*Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305; and †Department of Biochemistry and Walther Cancer Institute, Purdue University, West Lafayette, IN 47907

Contributed by Stanley Falkow, November 7, 1990

ABSTRACT **The plasmid-encoded YopH protein is a protein-tyrosine phosphatase (PTPase; EC 3.1.3.48) that is essential for *Yersinia* virulence. We have investigated the molecular basis for the role of PTPase activity in *Yersinia* pathogenesis. Allelic recombination was employed to introduce a defined mutation into the *yopH* plasmid gene. Conversion of the essential Cys-403 to Ala in the catalytic domain of the protein abolished YopH PTPase activity and significantly reduced the virulence of *Yersinia pseudotuberculosis* in a murine infection model. ^{32}P-labeled phosphotyrosine-containing proteins were immunoprecipitated from extracts of *Y. pseudotuberculosis*-infected cell monolayers and analyzed by SDS/PAGE to assess the impact of YopH on host protein phosphorylation. Major proteins of 200, 120, and 60 kDa were dephosphorylated in macrophages associated with wild-type *Y. pseudotuberculosis*. Selective removal of phosphate from the 120- and 60-kDa proteins was shown to be specific to the YopH PTPase activity. Phagocytosis of the bacteria was not required for this dephosphorylation activity, suggesting that YopH is functionally expressed by extracellular bacteria. These observations indicate that the essential function of YopH in *Yersinia* pathogenesis is host-protein dephosphorylation.**

Many bacterial pathogens have the capacity to infect a mammalian host and evade the ensuing innate and specific immune response. The expression of a set of proteins encoded on a common 70-kilobase (kb) virulence plasmid has been correlated with the capacity of pathogenic *Yersinia* to avoid mammalian host defense mechanisms (1). The synthesis and secretion of these proteins, termed Yops (*Yersinia* outer-membrane proteins), is coordinately regulated by two environmental signals encountered by the invading bacteria: a physiological temperature of 37°C and the submillimolar calcium concentrations typically found inside cells (2, 3). The *Yersinia pseudotuberculosis* proteins encoded by *yopE* and *yopH* have been identified as key virulence determinants (4, 5), and mutational loss of either gene has been reported to reduce the bacterium's antiphagocytic capabilities (6, 7).

The amino acid sequence of the *yopH* gene product was recently shown to contain significant homology to the conserved catalytic domains of the eukaryotic protein-tyrosine-phosphatase (PTPase; EC 3.1.3.48) family (8). The *yop51* gene, the homolog of *yopH* in *Yersinia enterocolitica* (9), was cloned and expressed in *Escherichia coli*. The recombinant enzyme has absolute specificity for removal of phosphate from peptides and proteins phosphorylated on tyrosine. A site-specific mutation was introduced into *yop51*; substitution of the conserved Cys-403 with Ala (C403A) abolished PTPase activity, demonstrating that this residue is essential for catalysis (8).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

The PTPase family is comprised of receptor-like and nonreceptor proteins that act alone or in concert with the protein-tyrosine kinases to modulate levels of cellular phosphorylation (10). Although the precise functions of individual PTPases are unknown, gross perturbations in PTPase activity can dramatically change the normal physiological properties of eukaryotic cells (11, 12). Regulation of PTPase activity is, therefore, crucial for animal cell function.

We reasoned that the pathogenic strategy of *Yersinia* includes the expression of a PTPase to disrupt host signal transduction processes involved in bacterial killing. We have tested this hypothesis by engineering a *Y. pseudotuberculosis* strain that is deficient in PTPase catalytic activity. Our results demonstrate that PTPase activity is essential for *Yersinia* virulence and indicate that the enzymatic activity of YopH is targeted toward host proteins.

MATERIALS AND METHODS

Bacterial Strains, DNA Isolations, and Techniques. The bacterial strains listed in Table 1 were grown and maintained on LB agar (GIBCO) plates or stored at −70°C in LB broth (GIBCO) containing 50% (vol/vol) glycerol. Overnight cultures were grown at 28°C with aeration. Techniques for large-scale preparations of plasmid DNA, filter hybridization, and molecular cloning were as described (15).

Plasmid Constructions. The plasmid used as a fusaric acid-selection vector (pFSV) was derived by replacement of the pACYC184 (16) origin of DNA replication with the origin of DNA replication and mobilization region from pJM703.1 (13). Briefly, the pACYC184 and pJM703.1 DNAs were cleaved with the restriction enzymes *Xba* I and *Eco*RI, respectively, and sequentially treated with S1 nuclease and the Klenow fragment of DNA polymerase I to generate blunt-ended molecules. The linearized DNAs were digested again with restriction enzymes that generate blunt ends: pACYC184 with *Xmn* I and pJM703.1 with *Hin*cII. The *Xba* I (blunt) to *Xmn* I DNA fragment of pACYC184 encoding resistance to tetracycline and chloramphenicol and the *Eco*RI (blunt) to *Hin*cII fragment containing the pJM703.1 replication and transfer functions were isolated and ligated together. The resulting plasmid is mobilizable and is stable only in strains expressing the R6K π protein (13). The *Eco*RI fragment containing the *yop51*C403A gene from the plasmid pT7-Yop51C403A (8) was ligated into the *Eco*RI site of pFSV to generate pFSVC403A.

Introduction of the C403A Mutation into pIB1. SM10λ pir harboring pFSVC403A was mated with YPIII(pIB1) on LB agar plates for 4 hr at 28°C. To select against both parental strains and to select for YPIII(pIB1) derivatives containing pFSVC403A, the mated bacteria were transferred to LB agar plates containing ampicillin (100 μg/ml) and tetracycline (10

Abbreviations: PTPase, protein-tyrosine phosphatase; cfu, colony-forming unit(s).

Table 1. Bacterial strains and plasmids

	Relevant properties	Ref. or source
Plasmid		
pJM703.1	*oriR6K mobRP4 ampR*	13
pFSV	*oriR6K mobRP4 tetR*	This study
pFSVC403A	pFSVyopHC403A	This study
Strain		
E. coli K-12		
SM10λpir	*recA::RP4pirR6K*	13
Y. pseudotuberculosis		
YPIII*	*ampR*	14
YPIII(pIB1)	*ampR*, pIB1$^+$	14
YPIII*inv*(pIB1)†	*inv*$^-$, pIB1$^+$	14
YPIII(pIB1C403A)	pIB1yopHC403A	This study
IP2666	Avirulent	M. Simonet
IP2666(pYV)	pYV$^+$, virulent	M. Simonet
IP2666(pIB1)	pIB1$^+$	This study
IP2666(pIB1C403A)	pIB1 yopHC403A	This study

*Designated YP201 in ref. 14.
†Designated YP212 in ref. 14.

μg/ml). To confirm in the exconjugants that pFSVC403A had integrated into pIB1, plasmid DNA was isolated, digested with *Bam*HI, and analyzed by agarose gel electrophoresis (1). Segregants having undergone a second recombination event leading to excision of the fusaric acid-selection vector sequences from pIB1 were isolated by fusaric acid selection (17, 18). Bacteria [10^6 colony-forming units (cfu)] from overnight cultures grown without tetracycline selection were plated on fusaric acid-containing agar plates and incubated at 28°C for 2–3 days. Fusaric acid-resistant colonies were tested for loss of tetracycline resistance and retention of pIB1 by replica plating onto LB agar plates containing tetracycline (10 μg/ml) and congo red/magnesium oxalate agar plates (19), respectively. Tetracycline-sensitive pIB1-containing isolates in which the C403A mutation was recombined into *yopH* were identified by screening for PTPase activity. Overnight bacterial cultures were subcultured 1:50 into a defined medium (20) supplemented with 1% tryptone, 0.5% yeast extract, 0.2% glucose, and 20 mM sodium oxalate and grown at 28°C to an OD_{600} of 0.3 followed by a shift to 37°C for 3 hr. Cells were harvested and assayed for PTPase activity as described (8). Secreted Yops were recovered from the culture supernatant (4) and analyzed by electrophoresis on a SDS/8–15% polyacrylamide gel. The *yopH* genes from pIB1 and pIB1C403A were amplified by the polymerase chain reaction (8), subcloned into Bluescript, and sequenced using a United States Biochemical Sequenase kit. To confirm the structure of the mutant gene *yopH*C403A, this procedure was repeated twice.

Mouse Virulence Assay. The virulence plasmids pIB1 and pIB1C403A were introduced into IP2666 by electroporation (21). To prepare bacteria for injection, overnight cultures of IP2666, IP2666(pIB1), and IP2666(pIB1C403A) were adjusted to an OD_{620} of 0.5, washed once in phosphate-buffered saline (PBS), and diluted to 10^4 cfu/ml in PBS. Six- to 8-week-old female Swiss mice were injected through the tail veins with 0.5 ml of diluted bacteria. For 1 week after the injection, the mice were monitored twice daily for signs of disease: ruffled fur, inappetence, and inactivity. Mice having all three symptoms were considered moribund and were sacrificed. Bacterial growth in the spleens of the injected mice was determined at intervals of 1–3 days as described (22).

Eukaryotic Cell Growth Conditions and Assays. The cells were grown and assayed at 37°C with 5% CO_2/95% air. J774A.1 mouse monocyte–macrophage cells (ATCC TIB 67) were propagated in Dulbecco's modified Eagle's medium containing 10% (vol/vol) fetal bovine serum. Hep-2 cells (14) were grown in RPMI 1640 medium containing 5% fetal bovine serum. To measure endogenous levels of protein-tyrosine phosphorylation, monolayers were prepared by seeding 5×10^5 cells in 5 ml into 60 × 15-mm tissue culture dishes and the cells were allowed to adhere overnight. The monolayers were rinsed with labeling medium (phosphate-free Eagle's minimum essential medium: GIBCO Select-Amine kit supplemented with 1% bovine serum albumin) and overlaid with 1 ml of labeling medium containing 100 μCi of [^{32}P]orthophosphate (Amersham; 1 Ci = 37 GBq). After 30 min, bacteria from overnight cultures (5×10^7 cfu) were added to the monolayers and incubated for an additional 3 hr. The infected monolayers were washed on ice once with PBS containing phosphatase inhibitors (0.4 mM EDTA/10 mM NaF/10 mM sodium pyrophosphate/0.4 mM sodium vanadate) and lysed in 1 ml of RIPA buffer (23). Identical methods were used for control (mock) infections without cultured cells. Phosphotyrosine-containing proteins were precipitated from the extracts by the addition of 1 μl (1 μg of IgG) of anti-phosphotyrosine monoclonal antibody (Upstate Biotechnical, Lake Placid, NY) and protein A-Sepharose CL-4B (Pharmacia) as described (23). The immune precipitates were fractionated on a SDS/8–15% polyacrylamide gel, and the fixed gel was dried and exposed to XAR-5 film at −70°C with an intensifying screen for 38 hr.

Entry of the bacteria into the cells was assayed as described (14) by measuring survival in the presence of gentamicin, an antibiotic that does not kill intracellularly localized bacteria. The monolayers were prepared as described for the dephosphorylation assay, except that 10^5 cells were seeded at 1 ml per well into 24-well tissue culture plates. Bacteria (10^7 cfu) were added to the monolayers incubating in labeling medium and allowed to bind and enter for 90 min. The medium overlaying the monolayers was then replaced with medium containing 100 μg of gentamicin sulfate per ml to kill extracellular bacteria. After 90 min, the monolayers were washed twice with PBS and lysed in 0.2 ml of Triton X-100 to release intracellular bacteria. Bacteria surviving gentamicin were quantitated by plating serial dilutions on LB agar plates.

RESULTS

Functional Expression of PTPase Activity in *Y. Pseudotuberculosis*. The *Y. pseudotuberculosis* serogroup III strain YPIII harbors a 70-kb virulence plasmid, designated pIB1 (4). We assayed YPIII(pIB1) and its isogenic plasmid-cured derivative YPIII for PTPase activity. To maximize YopH expression, whole-cell extracts were prepared from bacteria grown at 37°C in medium lacking calcium (4). As shown in Fig. 1*A*, dephosphorylation of a phosphotyrosine-containing peptide increased with increasing concentrations of extract from YPIII(pIB1). PTPase activity was not detected in the plasmid-free YPIII strain, indicating that expression of this phenotype is specific to the virulence plasmid.

Construction of the PTPase-Deficient *Y. pseudotuberculosis* Strain. By constructing a YPIII derivative identical in all respects to the wild-type strain except in PTPase activity, we could precisely focus on the role of PTPase activity in *Yersinia* pathogenesis. The sequences of *yopH* and *yop51* are 99% homologous (1393/1407 nucleotides shared) (9). This high degree of homology allowed us to introduce the C403A mutation from *yop51*C403A (8) into *yopH* by allelic recombination. The *yop51*C403A gene was inserted into a transferable and replication-deficient plasmid, and the codon substitution was incorporated into pIB1. The overall structure of the mutant plasmid designated pIB1C403A was verified by gel electrophoresis and filter hybridization using a probe homologous to *yopH* (Fig. 2). Wild-type pIB1 and the mutant pIB1C403A have indistinguishable *Bam*HI restriction patterns and are otherwise identical except for the specific

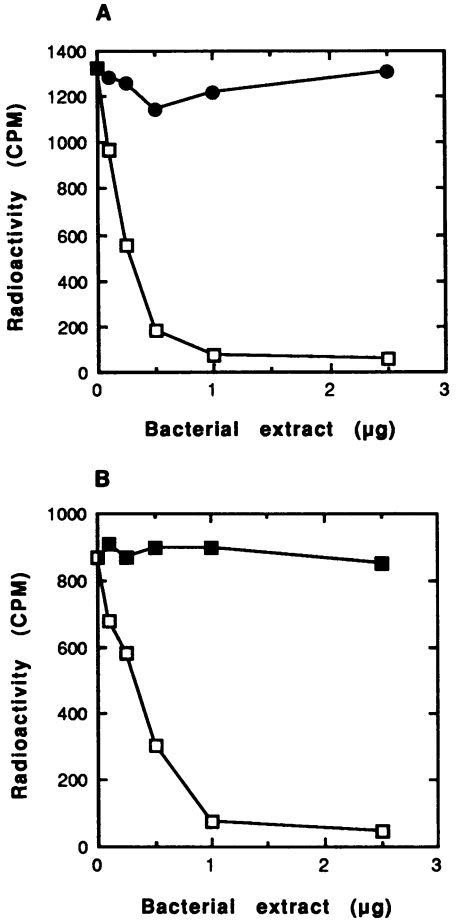

FIG. 1. Expression of PTPase activity in YPIII. Bacterial extracts were incubated with ^{32}P-labeled Raytide (Oncogene Science, Manhasset, NY) and after 10 min the mixtures were spotted onto phosphocellulose paper. Radioactivity remaining associated with the peptide was determined by scintillation counting after washing the filters. (*A*) Dephosphorylation of Raytide with extracts from YPIII (solid circles) and YPIII(pIB1) (open squares). (*B*) Dephosphorylation of Raytide with extracts from YPIII(pIB1) (open squares) and YPIII(pIB1C403A) (solid squares).

sequence differences introduced by recombination. The fine structure of *yopH*C403A was confirmed by sequencing, and except for the TGC (cysteine) to GCC (alanine) codon substitution at position 403 is identical to *yop51* (9).

Extracts were prepared from YPIII(pIB1) and YPIII-(pIB1C403A) and examined for PTPase activity. As shown in Fig. 1*B*, the C403A mutation abolished YopH PTPase activity. Analysis of the Yops secreted from YPIII(pIB1) and YPIII(pIB1C403A) by SDS/PAGE demonstrated that full-length (50 kDa) YopH is expressed equally in both strains (data not shown).

***Yersinia* Lacking PTPase Activity Are Avirulent.** Intravenous infection of mice was used to quantitate the effect of the C403A mutation on the virulence of *Y. pseudotuberculosis*. To ensure that any detected effects on virulence were specific to the introduced C403A mutation, pIB1 and pIB1C403A were purified from YPIII and transformed into the plasmid-cured derivative of *Y. pseudotuberculosis* IP2666(pYV), a highly virulent serogroup III strain. Mice were injected with IP2666, IP2666(pIB1), and IP2666(pIB1C403A), and bacterial growth in the spleen was determined for 1 week at intervals of 1–3 days (Fig. 3). IP2666(pIB1) multiplied rapidly, reaching 10^5 bacteria per spleen by day 4 and 10^6 bacteria per spleen by day 5. By day 5 the mice infected with IP2666(pIB1) were moribund and were sacrificed. In contrast, IP2666 and

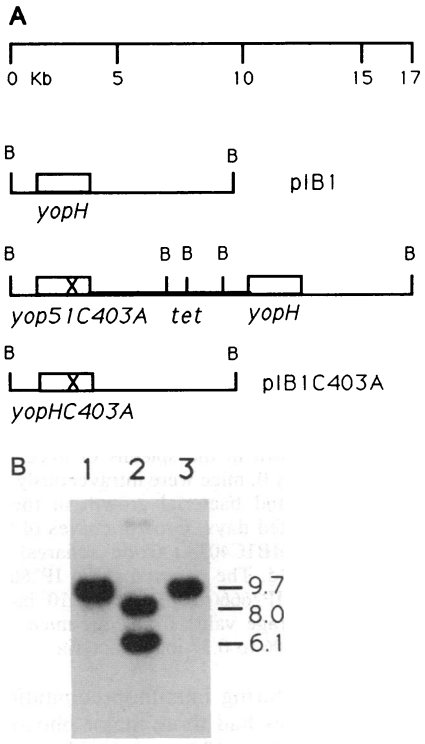

FIG. 2. Structure of *yopH* and the recombinant derivatives. (*A*) Restriction maps of pIB1-derived plasmids showing the *Bam*HI fragments encompassing *yopH* and the mutant derivatives of *yopH* generated by allelic recombination. B, *Bam*HI sites. Thin lines designate pIB1 sequences. The locations of the *yop* genes are shown by rectangles, with the TGC to GCC codon conversion (C403A) indicated by an X. Thick line designates the integrated fusaric acid-selection vector sequences. (*B*) Filter hybridization analysis of the sequences shown in *A*. Virulence plasmid DNA was isolated, digested with *Bam*HI, and analyzed by filter hybridization using as the probe the 1.4-kb *Eco*RI fragment of pT7-Yop51 (8) containing *yop51*. The probe hybridizes to a 9.7-kb *Bam*HI fragment encompassing *yopH* in the wild-type pIB1 (lane 1) and *yopH*C403A in the mutant pIB1C403A (lane 3) plasmids and to 8.0-kb and 6.1-kb fragments in the cointegrate plasmid (lane 2).

IP2666(pIB1C403A) grew at a slower rate, reaching a maximum of 10^4 bacteria per spleen at day 4. The mice infected with IP2666(pIB1C403A) had survived the bacterial challenge by day 7 with only mild disease symptoms. The IP2666-infected mice remained asymptomatic throughout the course of the experiment. These observations indicate that the intrinsic PTPase activity of YopH is essential for YPIII to cause disease in this animal model.

Host Proteins Are Dephosphorylated by the *Yersinia* PTPase. To investigate the possibility that the PTPase activity of YopH is targeted toward host proteins, we measured changes in endogenous levels of tyrosine phosphorylation associated with the binding and phagocytosis of YPIII by a murine macrophage-like cell line, J774A.1 (24). Phosphorylated proteins were radioactively labeled by the addition of [^{32}P]orthophosphate to macrophage monolayers. The macrophages were simultaneously infected with the YPIII, YPIII(pIB1), and YPIII(pIB1C403A) strains that had been grown at 28°C without calcium limitation [conditions that repress expression of YopH (4)]. Phagocytosis of YPIII by the macrophages was documented by measuring survival in the presence of gentamicin, an antibiotic that does not kill intracellular bacteria (Table 2). Phosphotyrosine-containing proteins were immunoprecipitated from total cell extracts with an anti-phosphotyrosine monoclonal antibody and analyzed by SDS/PAGE (Fig. 4*A*). The specificity of the monoclonal antibody was proven by the addition of excess phosphoty-

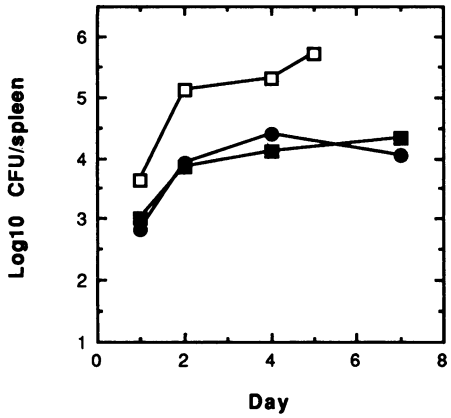

FIG. 3. Bacterial growth in the spleens of mice infected with IP2666 derivatives. On day 0, mice were intravenously injected with bacteria (2×10^3 cfu) and bacterial growth in the spleen was determined on the indicated days. Growth curves of IP2666(pIB1) (open squares), IP2666(pIB1C403A) (solid squares), and IP2666 (solid circles) are indicated. The parental strain IP2666 (pYV) had growth kinetics similar to IP2666(pIB1) reaching 10^5 bacteria by day 4. Each point is the average value from four mice; the standard deviations ranged from 0.06 to 0.37 $\log_{10}$ bacteria.

rosine to one extract during immunoprecipitation (lane 5). Uninfected macrophages had three major phosphotyrosine-containing proteins of 200, 120, and 60 kDa (lane 1). The 200-kDa protein was dephosphorylated in response to infection by all three strains (lanes 2–4). This alteration appears to reflect a macrophage-mediated event in response to phagocytic activity. Additional changes in protein phosphorylation were mediated only by wild-type bacteria. Interaction of YPIII(pIB1) with the macrophages lead to significant (5-fold as quantitated by densitometry) dephosphorylation of the 120- and 60-kDa proteins (lane 3). Dephosphorylation of the 120- and 60-kDa proteins was due to the activity of YopH, since association of YPIII(pIB1C403A) with the macrophages did not cause removal of tyrosyl phosphate from these proteins (lane 4).

From this result, we can conclude that YopH acts on multiple substrates in the cytoplasm of the host cell. To reach these substrates, YopH must first be expressed and exported by the bacteria and then translocated across the host membrane. To determine if intracellular localization is required for the first step of this process, we performed an additional experiment in which bacteria were allowed to bind but not enter host cells.

Efficient entry of YPIII into epithelial cells requires the function of invasin, a 103-kDa surface protein that binds to an integrin receptor of the VLA class to promote bacterial uptake (25). In accordance with previous results (14), loss of

Table 2. Entry of YPIII derivatives into cultured cells

Cell type	Bacterial strain	% phagocytosed
J774A.1		
	YPIII	3.42 ± 0.14
	YPIII(pIB1C403A)	3.40 ± 0.53
	YPIII(pIB1)	2.55 ± 0.12
Hep-2		
	YPIII	0.75 ± 0.10
	YPIII(pIB1C403A)	1.45 ± 0.08
	YPIII(pIB1)	1.16 ± 0.12
	YPIII*inv*(pIB1)	0.073 ± 0.009

Strains were grown at 28°C and added to J774A.1 or Hep-2 monolayers to quantitate bacterial entry. Percent phagocytosed was quantitated as percentage of organisms surviving gentamicin treatment relative to the number of added bacteria. Results are from three determinations (mean ± SD).

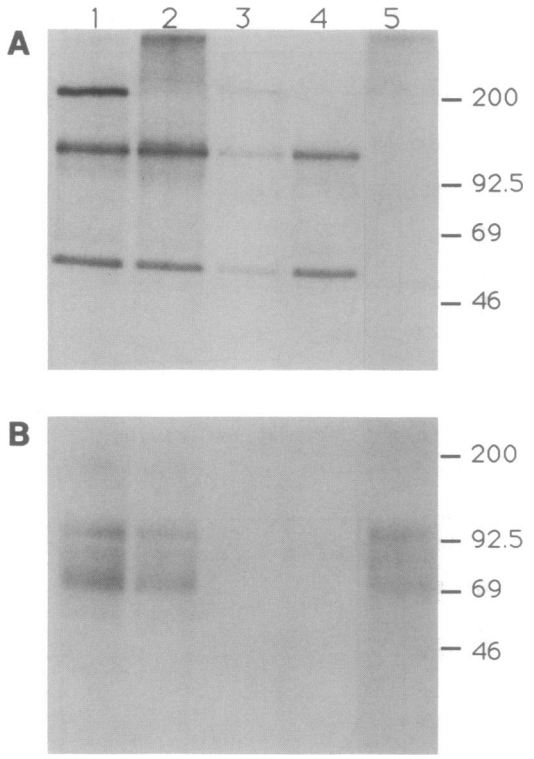

FIG. 4. Dephosphorylation of host cell proteins by the *Yersinia* PTPase. Monolayers were incubated in phosphate-free tissue culture medium and [^{32}P]orthophosphate to radioactively label phosphorylated proteins. Phosphotyrosine-containing proteins from total cell extracts were immunoprecipitated with an anti-phosphotyrosine monoclonal antibody and analyzed by SDS/PAGE. (*A*) Dephosphorylation of macrophage proteins. Uninfected macrophages (lane 1), macrophages infected with YPIII (lane 2), with YPIII(pIB1) (lane 3), or with YPIII(pIB1C403A) (lane 4) are shown. To demonstrate the specificity of the monoclonal antibody, *O*-phospho-L-tyrosine (10 mM) was included in one extract prior to immunoprecipitation (lane 5). (*B*) Dephosphorylation of Hep-2 proteins. Proteins from uninfected cells (lane 1) or cells infected with YPIII (lane 2), with YPIII(pIB1) (lane 3), with YPIII*inv*(pIB1) (lane 4), or with YPIII(pIB1C403A) (lane 5) are shown. Each lane contains the phosphotyrosine-containing proteins precipitated out of extracts made from equivalent numbers (5×10^5) of macrophage or Hep-2 cells.

invasin function reduced the entry of YPIII*inv*(pIB1) into epithelial (Hep-2) cells by 94% (Table 2). In Hep-2 cells associated with either inv^+ or inv^- pIB1-containing bacteria, major proteins of 100 and 70 kDa were specifically dephosphorylated by YopH, indicating that invasin-mediated phagocytosis of the bacteria was not required for YopH expression (Fig. 4*B*). Although these data suggest that YopH is functionally expressed by extracellular bacteria, this experiment does not rule out the possibility that the observed activity originated from very low numbers of bacteria entering by an alternative pathway (14).

DISCUSSION

We have constructed a *Y. pseudotuberculosis* strain that expresses a catalytically inactive form of YopH. Previous reports have shown that mutational loss of YopH reduced the virulence of the three *Yersinia* species that are pathogenic for mammals (4, 9, 20). Analysis of our single-site mutant in a murine infection model demonstrated that the essential feature of this protein is functional PTPase activity (Fig. 3).

This capacity of YopH to promote virulence is most likely a direct result of its tyrosine phosphate hydrolysis activity in host cells. We analyzed the phosphotyrosine-containing pro-

teins of host cells associated with YPIII(pIB1) and observed dephosphorylation of multiple eukaryotic proteins in a YopH-dependent process (Fig. 4). We have begun to investigate the process of YopH expression using mutations (14) that reduce *Yersinia* entry into host cells. Whether YopH can be expressed by extracellular bacteria, as our data suggest, or only in a phagosomal compartment, the protein must cross a host membrane to be functional. The Yersiniae encode a specialized, calcium-regulated pathway for export of YopH and the other Yops; secretion of these plasmid-encoded proteins does not depend on typical signals (26). It remains to be determined if YopH is capable of independent translocation through the eukaryotic membrane, if additional cofactors are required, or if the secretion process is mediated by bacterial attachment to host cell receptors.

The idea that YopH is functionally expressed by extracellular bacteria once its secretion is induced can be correlated with the cytological observations of the infection process in animal models. We believe that the enteropathogenic *Yersinia* initially replicate intracellularly in the Peyer's patches of the terminal ileum. In the mouse, the bacteria subsequently proliferate predominantly in extracellular niches within the lymphoid tissues and are rarely detected within phagocytic cells (22). The phagosomal environment encountered by the intracellular bacteria early in infection would positively activate the expression and secretion of the Yops (27), which may condition the bacteria for extracellular survival in the potentially hostile environment of the reticuloendothelial system.

Phagocytosis, antigen presentation, and activation are fundamental macrophage processes involved in bacterial killing (28). Because these functions are modulated in large part by external stimuli and signal transduction, they are potentially key targets of YopH. Mutational loss of YopH was shown (6), under specific conditions not used in this study, to increase the rate of YPIII phagocytosis by macrophages *in vitro*. This phenomenon can now be understood in terms of the molecular basis for YopH activity. An even better understanding of the overall impact of YopH on immune cell function will follow from the identification of the precise proteins in phagocytic cells that are selectively dephosphorylated by this enzyme.

Because of their need to overcome similar host barriers, common mechanisms of microbial pathogenesis have evolved in distinct bacterial species (29). Since bacteria do not generally contain tyrosine phosphate, the *Yersinia* PTPase might be descended from a host gene, a situation ascribed to the derivation of retroviral-encoded tyrosine kinases (30).

This work was supported by Public Health Service Grant AI26195 (to S.F.), National Institute of Diabetes and Digestive Kidney Diseases Grant 18849, and Walther Cancer Institute Grants (to J.E.D. and K.G.). J.B.B. was supported by a National Institute of Allergy and Infectious Diseases Fellowship AI07851-01.

1. Portnoy, D. A., Wolf-Watz, H., Bolin, I., Beeder, A. B. & Falkow, S. (1984) *Infect. Immun.* **43,** 108–114.
2. Brubaker, R. R. (1983) *Rev. Infect. Dis.* **5,** S748–S758.
3. Cornelis, G., Laroche, Y., Balligand, G., Sory, M. P. & Wauters, G. (1987) *Rev. Infect. Dis.* **9,** 64–87.
4. Bolin, I. & Wolf-Watz, H. (1988) *Mol. Microbiol.* **2,** 237–245.
5. Forsberg, A. & Wolf-Watz, H. (1988) *Mol. Microbiol.* **2,** 121–133.
6. Rosqvist, R., Bolin, I. & Wolf-Watz, H. (1988) *Infect. Immun.* **56,** 2139–2143.
7. Rosqvist, R., Forsberg, A., Rimpilainen, M., Bergman, T. & Wolf-Watz, H. (1990) *Mol. Microbiol.* **4,** 657–667.
8. Guan, K. & Dixon, J. E. (1990) *Science* **249,** 553–556.
9. Michiels, T. & Cornelis, G. (1988) *Microb. Pathog.* **5,** 449–59.
10. Hunter, T. (1989) *Curr. Opin. Cell Biol.* **1,** 1168–1181.
11. Cicirelli, M. F., Tonks, N. K., Diltz, C. D., Weiel, J. E., Fischer, E. H. & Krebs, E. G. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 5514–5518.
12. Klarlund, J. K. (1985) *Cell* **41,** 707–717.
13. Miller, V. & Mekalanos, J. (1988) *J. Bacteriol.* **170,** 2575–2583.
14. Isberg, R. R. (1989) *Infect. Immun.* **57,** 1998–2005.
15. Maniatis, T., Fritsch, E. & Sambrook, J. (1982) in *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
16. Chang, A. C. & Cohen, S. N. (1978) *J. Bacteriol.* **134,** 1141–1156.
17. Maloy, S. R. & Nunn, W. D. (1981) *J. Bacteriol.* **145,** 1110–1111.
18. Bochner, B. R., Huang, H. C., Schieven, G. L. & Ames, B. N. (1980) *J. Bacteriol.* **143,** 926–933.
19. Riley, G. & Toma, S. (1989) *J. Clin. Microbiol.* **27,** 213–214.
20. Straley, S. C. & Bowmer, W. S. (1986) *Infect. Immun.* **51,** 445–454.
21. Dower, W. J., Miller, J. F. & Ragsdale, C. W. (1988) *Nucleic Acids Res.* **16,** 6127–6145.
22. Simonet, M., Richard, S. & Berche, P. (1990) *Infect. Immun.* **58,** 841–845.
23. Harlow, E. & Lane, D. (1988) *Antibodies: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
24. Ralph, P. & Nakoinz, I. (1975) *Nature (London)* **257,** 393–394.
25. Isberg, R. R. & Leong, J. M. (1990) *Cell* **60,** 861–871.
26. Michiels, T., Wattiau, P., Brasseur, R., Ruysschaert, J. & Cornelis, G. (1990) *Infect. Immun.* **58,** 2840–2849.
27. Pollack, C., Straley, S. C. & Klempner, M. S. (1986) *Nature (London)* **322,** 834–836.
28. Adams, D. O. & Hamilton, T. A. (1984) *Annu. Rev. Immunol.* **2,** 283–318.
29. Finlay, B. B. & Falkow, S. (1989) *Microbiol. Rev.* **53,** 210–230.
30. Bishop, J. M. (1987) *Science* **235,** 305–311.